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# Effect of LED vs. fluorescent light on rat pineal melatonin and retinal function

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**EFFECT OF LED VS. FLUORESCENT LIGHT ON RAT PINEAL MELATONIN AND  
RETINAL FUNCTION**

**A Thesis**

**Presented to**

**The Faculty of the Department of Biology**

**San Jose State University**

**In Partial Fulfillment**

**of the Requirements for the Degree**

**Master of Science**

**by**

**Darren Scott Heeke**

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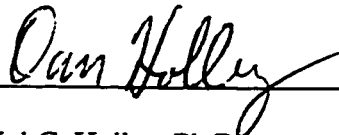
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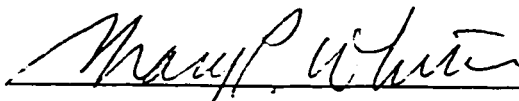
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## ABSTRACT

### EFFECT OF LED VS. FLUORESCENT LIGHT ON RAT PINEAL MELATONIN AND RETINAL FUNCTION

by Darren S. Heeke

The purpose of this study was to evaluate a novel LED light source for use in animal habitat lighting by comparing its effectiveness to cool-white fluorescent (CWF) light in suppressing pineal gland melatonin (M) and maintaining normal retinal physiology and morphology in the rat. M suppression experiments showed that LED light suppressed pineal M equally to that of CWF light at five different light intensities (100, 40, 10, 1 and 0.1 lux). There were no significant differences in M suppression between LED and CWF when compared to unexposed controls ( $p > .08$ , ANOVA). Retinal physiology was evaluated using electroretinography. There were no significant differences in ERG a-wave implicit times and amplitudes and b-wave implicit times and amplitudes between LED-exposed rats and CWF-exposed rats ( $p > .09$ , ANOVA). Results of retinal histology assessment show there is no difference in retinal thickness, rod outer segment length and number of rod nuclei between LED-exposed rats and CWF-exposed rats ( $p > .42$ , ANOVA).



## ACKNOWLEDGMENTS

Supported by NASA Cooperative Agreement NCC2-779 to Dr. D.C. Holley. I thank Ms. Xuan Sarah-Khanh Duong for her assistance with the retinal histology procedures and analysis as well as Dr. Sulekha Anand for her assistance with the statistical analysis. I also thank Dr. George Brainard, John Hanifin, and Dr. Mark Rollag for their support and consultation throughout this project. In addition, I extend considerable thanks to Dr. Daniel C. Holley, Dr. Robert Hyde, Dr. Mary P. White and Dr. Charles Winget for their continuous guidance and support during this project as well as their meticulous review of the manuscript. Finally, I thank my wife Lori for her patience and support during this entire process.

## INTRODUCTION

The profound effect of light on circadian physiology is well established (1). It is also well established that under certain conditions, light can be phototoxic to the retina (2,3,4). However, the 1985 edition of the Guide for the Care and Use of Laboratory Animals stated, "Precise lighting requirements for maintenance of good health and physiological stability of animals are not known" (5). Needing exact engineering specifications for animal habitat construction for microgravity studies, NASA sponsored a working group who recommended relatively precise intensity and spectral power specifications for husbandry of rodents and non-human primates (6). The 1996 edition of the Guide for the Care and Use of Laboratory Animals indicated that intensity, duration of exposure, and wavelength are among the factors that should be considered when appropriate illumination is being considered for animal holding room lighting (7). The Guide specifies intensity standards for the albino rat, but does not specify standards for photoperiod or wavelength. The Guide also warns about albino rats vulnerability to high light intensity-induced retinal phototoxicity. Currently, when discussing light intensity in human or animal environments, the term "luminous flux" (known as lux) is used, which refers to the amount of light falling on a surface that stimulates the human eye during the daytime. Lux is the standard unit used for specifying lighting for buildings, including buildings which house animals (7).

Currently, the light source most commonly used in animal vivariums is broad-band fluorescent white light, sometimes referred to as cool white fluorescent (CWF) light.

NASA uses incandescent bulbs in the space shuttle Animal Enclosure Module (AEM) and the Research Animal Holding Facility (RAHF) which require more energy than fluorescent lamps and produce considerable heat. Light emitting diode (LED) technology offers a relatively inexpensive alternative light source and has inherent advantages including: spectral control, high energy efficiency, long operating life, low heat production, ruggedness (solid state), and certain mechanical/size advantages (8).

As a measure of neuroendocrine function, we compared the effectiveness of LED light to CWF light in suppressing pineal gland melatonin. To establish whether LEDs cause functional retinal damage, we compared the effects of LED light and CWF light on retinal function using electroretinography. We also examined effects of LED light and CWF light on retinal morphology using light microscopy.

## **MATERIALS AND METHODS**

### **Light Sources and Measurement**

Most commercially available LEDs are individual bulbs that emit restricted bandwidths of light giving distinct color appearances. Groups of monochromatic LEDs, however, can be arranged so as to approximately match the spectral power distribution of CWF light. The LED light source used in these experiments consisted of circular (8-inch diameter), honeycomb arrays of blue, green, yellow and red LEDs (blue LED, catalog # C470-5C18, Cree Research Inc., Durham, NC; green LED, catalog # HLMP-3950, Future Active Industrial Electronics, San Jose, CA; yellow LED, catalog # HLMP-3850, and red

LED, catalog # HLMP-3750, Hamilton/Halmark, Tempe, AZ). These were arranged and powered to give approximately the same spectral power distribution as CWF light. Spectral power distributions were measured using a calibrated radiometer (Model IL-1700, International Light Inc., Newburyport, MA) with a scanning spectral radiometer attachment (Model IL-780/SHD033, International Light Inc., Newburyport, MA). Figure 1 shows the spectral power distribution curves for the 100 lux CWF and LED light sources used in this study.

In the melatonin suppression study, illuminances at animals' eye level were measured with a Minolta illuminance meter (Model T-1, Osaka, Japan). Once illuminance was set it was measured with a J16 radiometer and a remote J6512 probe (Tektronix, Inc., Beaverton, OR). The CWF experimental light was produced by an 8-watt CWF bulb (catalog # F8T5, Philips Lighting, Kanagawa, Japan). The LED experimental light array used in this experiment was modified from the original 8-inch diameter, circular, honeycomb array to a more condensed 6-inch diameter circular pattern. The tighter packing of LEDs helped increase the overall maximum illuminance of the LED array. Light from the LED array was also concentrated by use of a fresnel lens (Model A32681, Edmund Scientific Company, Barrington, NJ) in all exposures. Glass neutral density filters (Oriel Corp., Stratford, CT) were used to adjust the intensity of both experimental lights. The duration of the light pulse was automatically controlled by a variable timer (Solar Light Co., Philadelphia, PA) attached to an iris diaphragm.

In the retinal phototoxicity study, illuminances at animals' eye level were measured using a calibrated radiometer (Model IL-1700, International Light Inc., Newburyport, MA) with a photometer sensor (Model # SED038, Serial # 2064 using Y filter # 8990 and W diffuser # 4945, International Light Inc., Newburyport, MA). Direct energy was measured (in  $\mu\text{W}/\text{cm}^2$ ) with the same calibrated radiometer and an irradiance sensor (Model # SED038, Serial # 2143 using F filter #18629 and W diffuser #9089, International Light Inc., Newburyport, MA). The range of illuminances produced by the photic stimulus during the electroretinographic assessment was measured using the same calibrated radiometer and the photometer sensor.

### **Melatonin Suppression Study**

All experiments performed in this study were reviewed and approved by Jefferson Medical College Institutional Animal Care and Use Committee and San Jose State University Institutional Animal Care and Use Committee to assure that experiments minimized any potential pain and discomfort of experimental animals. Adult male Sprague Dawley rats (175-200g, Harlan Sprague Dawley Breeding Laboratories, Indianapolis, IN) were maintained on a 12:12 light:dark (LD) cycle (lights on 0600-1800 h) for a minimum of 2 weeks before experimentation. The daily light cycle was produced by 40-watt CWF light sources (catalog # F40-CW, Sylvania, Danvers, MA), which provided an illuminance of  $60 \pm 6$  lux ( $18 \pm 2 \mu\text{W}/\text{cm}^2$ ) at the animal's eye level. Animal handling was carried out under dim red light produced by a 25-watt incandescent bulb

behind a Kodak 1A safelight filter (Eastman Kodak, Rochester, NY). The red light had an illuminance of 0.4 - 1.5 lux (20-80  $\mu\text{W}/\text{cm}^2$ ).

Each of the melatonin suppression experiments took place between 0000 h and 0230 h on separate days. Each experiment included 3 sets of eight rats: a group exposed to CWF light, a group exposed to the LED array and an unexposed control group. Each exposure lasted 5 min and occurred while the rats were dark-adapted. Five different light illuminances were tested: 100 lux, 40 lux, 10 lux, 1.0 lux, and 0.1 lux which correspond to the following LED and CWF irradiances, respectively: 32.0  $\mu\text{W}/\text{cm}^2$  and 34.8  $\mu\text{W}/\text{cm}^2$ , 11.5  $\mu\text{W}/\text{cm}^2$  and 13.0  $\mu\text{W}/\text{cm}^2$ , 3.40  $\mu\text{W}/\text{cm}^2$  and 3.23  $\mu\text{W}/\text{cm}^2$ , 0.30  $\mu\text{W}/\text{cm}^2$  and 0.36  $\mu\text{W}/\text{cm}^2$ , 0.036  $\mu\text{W}/\text{cm}^2$  and 0.044  $\mu\text{W}/\text{cm}^2$ . Rats were individually exposed to their respective light source (LED or CWF) for 5 min in a 18.5 x 10.5 x 12.5 cm flat black box. The exposure system was a modification of an apparatus described elsewhere (9,10). Following the 5-min light exposure, rats were transferred to a light-tight holding box where they were held in darkness for 15 min. Rats were then euthanized by decapitation and pineal glands were removed, placed in a microtiter plate and frozen at -20 °C.

The pineal glands were later assayed for melatonin content using a modification of the radioimmunoassay (RIA) described by Rollag and Niswender (11). In this procedure, pineal glands were suspended in 200  $\mu\text{L}$  PBS-0.1% gelatin and dispersed with a microultrasonic cell disrupter (Kontes, Vineland, NJ). Duplicate 20- $\mu\text{L}$  samples of the sonicate were each placed into an additional 180  $\mu\text{L}$  PBS-0.1% gelatin. Each duplicate

(200  $\mu\text{L}$  volume) was then incubated with 100  $\mu\text{L}$  of [ $^{125}\text{I}$ ] (40,000cpm/100  $\mu\text{L}$  or 57,000dpm/100  $\mu\text{L}$ ) melatonin analog and 100  $\mu\text{L}$  of a 1:64,000 dilution of rabbit antiserum (antibody raised by Mark D. Rollag on 9/16/74 in rabbit # R1055) to give a final volume of 400  $\mu\text{L}$ . After a 2-day incubation at 4°C, antibody-bound radioactivity was precipitated by adding 3mL cold (4°C) 96% ethanol to the incubation mixture and centrifuging at 1000 x g for 30 min. The intra- and interassay coefficients of variation were less than 10% each. Each melatonin assay included an unexposed control group, an LED-exposed group and a CWF-exposed group from one light illuminance cohort.

For each study, all data from the CWF-exposed, LED-exposed and control animals were compared by analysis of variance (ANOVA), where differences were considered significant if  $p \leq .05$ . Specific comparisons between sets of animals were assessed by Scheffe tests.

### **Retinal Phototoxicity Study**

All experiments performed in this study were reviewed and approved by the San Jose State University Institutional Animal Care and Use Committee to assure that experiments minimized any potential pain and discomfort of experimental animals. In the retinal phototoxicity study, the animals were housed under either LED lighting or CWF lighting. Sixteen male Sprague Dawley rats (300-350g, Simonsen Laboratories, Gilroy, CA) were used in this experiment and were housed and tested together in groups of four: two control animals (CWF-exposed rats) and two experimental animals (LED-

exposed rats). CWF-exposed rats were housed in a chamber fitted with two 14-watt CWF bulbs (catalog # GE F14T12, GE Lighting, Cleveland, OH). LED-exposed rats were housed in a separate chamber fitted with two LED arrays. Individual rats were kept in shoebox-type cages fitted with aluminum sheet metal shaped in a cylindrical ring (8 inches in diameter and 8 inches in height). This design helped provide uniform light exposure to the animal as well as an unobstructed light path. The light sources were mounted from the ceilings of each of the chambers, to ensure that each animal would be exposed directly from overhead with one CWF bulb or LED panel. The desired CWF irradiance was produced by masking the CWF bulbs with aluminum foil as well as adjusting the distance between the CWF bulbs and the rat cages. The desired LED irradiance was established by adjusting the distance between the LED panel and the rat cages. The illuminance used in this study was 100 lux (corresponding irradiances were  $28.5 \mu\text{W}/\text{cm}^2$  from the LED panel and  $30.1 \mu\text{W}/\text{cm}^2$  from the CWF source). The two exposure chambers, one exposing two rats to CWF fluorescent light and the other exposing two rats to LED light, were both constructed and maintained so as to keep external light from entering.

Rats were exposed to their respective light source using 12:12 LD cycles for 14 days, with lights on at 0700 hours. Both the duration of exposure and the cyclic lighting schedule are important factors when determining the extent of light-induced retinal damage (12). According to some studies, light cycles with greater than 12 hours of light per day significantly increase the damaging effects of light on the retina (13). Also, it has



been found that quantitative changes in morphology of the retina following toxic light exposure are most reliably measured approximately 14 days after exposure (14). All animal handling and maintenance was done under dim red lighting (Kodak Safelight #2 - Eastman Kodak, Rochester, NY).

### **Electroretinography**

On the fifteenth day of the retinal phototoxicity study, rats were moved to a darkroom following dark adaptation overnight (the last dark cycle of the 14<sup>th</sup> day) for scotopic electroretinography (ERG) assessment. Ninety five percent of the rat retina is comprised of rod photoreceptor cells that are best studied in dark adaptation (15). Electroretinography was performed on the rats in the morning no earlier than 0930 hours to avoid variability associated with photoreceptor disc shedding (16).

The rats were injected i.p. with urethane (0.15g/kg body weight; 20% aqueous solution). Pupils were dilated with 10% phenylephrine eye drops. After anesthesia, the rat's eyelid was held open and the cornea was protected from drying with methylcellulose drops. A photostimulator producing white light (Grass PS22 Photo Stimulator, Grass Medical Instruments, Quincy, MA) was used as a stimulus control to confirm an ERG response in the rats. Monocular recordings were obtained using a 3mm diameter loop of platinum-iridium wire as the recording electrode on the cornea, a reference electrode on the forehead and a ground electrode on the cheek. The signals were AC-amplified with 0.1-kHz (low frequency) and 1-kHz (high frequency) cutoffs and displayed on a PC

monitor for cursor placement and amplitude measurement, using the computerized BPM-100 ERG recording system (RetinoGraphics, Inc., Norwalk, CT). The photic stimulus used in the ERG recordings was a blue LED light placed a standard distance of 9 cm above the eye to illuminate the entire retina. ERG responses to a series of brief flashes varying in intensity were recorded, starting at the highest intensity and stepping down to the lowest intensity. Each step in intensity represented a  $\frac{1}{2}$ -log unit. The range of illuminance of the brief flashes was from a maximal 130 lux to 0.1 lux. The range of light intensity was controlled by the BPM-100 ERG recording system which adjusts the power of the stimulus electrically rather than by placement of neutral density filters in the path of the light.

The average of eight responses was obtained at each intensity, with an interstimulus interval of 20 seconds. Later the ERG waveforms were measured using cursor placement to determine the a- and b-wave amplitudes and implicit times. These values were plotted against the stimulus intensity (intensity-response functions). Statistical evaluation of the data used analysis of variance (two way-ANOVA), where differences were considered significant if  $p \leq .05$ . Stimulus intensity was confirmed by direct energy measurement, at the beginning of the experiment and at the end of the experiment. Upon completion of ERG data collection, each rat was sacrificed with CO<sub>2</sub> gas. The right eyes were removed and placed in modified Karnovsky's fixative for later histological preparation and analysis.

## **Retinal Histology**

To determine whether light damage occurred in the retinas of the LED and CWF-exposed rats of the retinal phototoxicity study, the retinal epithelium, rod outer segments, rod nuclei, and total retinal thickness were inspected. The quantitative histological analysis was carried out on 7 LED-housed rats from the ERG study, 7 of the CWF-housed rats from the ERG study, as well as 3 control rats that were not subjected to the ERG procedure. The 3 control rats were not exposed to the experimental chamber environments, but maintained on a 12:12 LD cycle of CWF light (250 lux, lights on at 0700) in an animal vivarium for 14 days. Eyes were fixed by immersion in modified Karnovsky's fixative (1% paraformaldehyde, 1.5% glutaraldehyde in cacodylate buffer) for at least 72 hours. Following fixation, each eye was postfixed in 2% osmium tetroxide, bisected on the vertical meridian and embedded in epon-araldite plastic.

The retinal tissue was sectioned with a Sorval JB-4 ultramicrotome (Dupont Instruments). Semi-thin sections were stained with toluidine blue (1% in 1% borax) and were examined on a Nikon research microscope with an ocular micrometer attachment. Retinal measurements were made under 100X oil immersion at 270 microns and 450 microns from the optic nerve head.

The retinal pigmented epithelium was inspected for the presence of missing areas of retinal pigmented epithelium, vacuolation and vesicles. The rod outer segment length

was measured and observed for any signs of damage and disorganization. The number of rod nuclei were counted perpendicular to the retinal pigmented epithelium. Lastly, the thickness of the retina was measured perpendicular to the retinal pigmented epithelium. Statistical evaluation of the data used one-way ANOVA, where differences were considered significant if  $p \leq .05$ .

## RESULTS

### Melatonin Suppression Study

Figure 2 shows the pineal melatonin concentrations for each of the 3 groups of rats at their respective light exposure intensities. For the 100-lux intensity experiment, the control, CWF, and LED groups showed pineal melatonin concentrations of  $1166 \pm 136$ ,  $393 \pm 41$ , and  $439 \pm 25$  pg/gland (mean  $\pm$  SEM), respectively. For the 40-lux intensity experiment, the control, CWF, and LED groups showed pineal melatonin concentrations of  $1569 \pm 126$ ,  $365 \pm 34$ , and  $462 \pm 50$  pg/gland (mean  $\pm$  SEM), respectively. For the 10-lux intensity experiment, the control, CWF, and LED groups showed pineal melatonin concentrations of  $353 \pm 34$ ,  $257 \pm 13$ , and  $231 \pm 6$  pg/gland (mean  $\pm$  SEM), respectively. For the 1.0-lux intensity experiment, the control, CWF, and LED groups showed pineal melatonin concentrations of  $650 \pm 124$ ,  $218 \pm 42$ , and  $164 \pm 12$  pg/gland (mean  $\pm$  SEM), respectively. For the 0.1-lux intensity experiment, the control, CWF, and LED groups showed pineal melatonin concentrations of  $464 \pm 85$ ,  $239 \pm 71$ , and  $158 \pm 12$  pg/gland (mean  $\pm$  SEM), respectively.

In both experimental light conditions, at all illuminances examined, the rats showed significant melatonin suppression (100 lux:  $p < .0001$ ; 40 lux:  $p < .0001$ ; 10 lux:  $p < .002$ ; 1.0 lux:  $p < .001$ ; 0.1 lux:  $p < .01$ , respectively). In no case was the melatonin suppression induced by an LED illuminance significantly different from the melatonin suppression elicited by the same illuminance of CWF light (100 lux:  $p > .35$ ; 40 lux:  $p > .13$ ; 10 lux:  $p > .08$ ; 1.0 lux:  $p > .23$ ; 0.1 lux:  $p > .28$ , ANOVA).

### **Retinal Phototoxicity Study**

No evidence of retinal phototoxicity was found in the ERG responses from rats housed in LED or CWF light. Examples of individual ERG waveforms are shown in Figure 3. ERG response-intensity curves are shown (mean  $\pm$  1 S.D.) for both exposure groups in Figures 4 - 7. Figures 4 and 5 plot the a-wave implicit times and amplitudes for the LED and CWF groups. Figures 6 and 7 plot the b-wave implicit times and amplitudes for the LED and CWF groups. ERG a-waves were rarely observed in the lower photic stimulus intensities and were undetectable below the  $-3/2$  log intensity in both LED and CWF animals. The mean implicit times and amplitudes of the ERG a- and b-waves are not significantly different for the two treatment/exposure groups ( $p > .09$ , two-way ANOVA).

## **Retinal Histology**

No evidence of phototoxicity was found in retinal morphology. Table 1 shows the results of the quantitative measurements taken on the rat retinas. The retinas, including the retinal pigmented epithelium and rod outer segments, of all the eyes were in good condition and their thickness was normal. No significant differences were found between LED and CWF groups in the measurements of rod outer segment length, number of outer nuclear layer nuclei, or overall thickness of the retina ( $p > .42$ , one-way ANOVA).

## **DISCUSSION**

This study shows that a novel LED light source can suppress pineal gland melatonin concentrations as well as a conventional CWF light source at similar intensity levels. Also, we have demonstrated that there are no significant differences in retinal function (determined by ERG) or histology for animals exposed to LEDs compared to animals exposed to CWF lighting.

Investigating the effects of light on mammalian physiology involves the discipline of photobiology, the interaction between optical radiation and living organisms. Specifically, the science of photobiology is the study of how the infrared, visible and ultraviolet portions of the electromagnetic spectrum influence biological processes (17,18). Light, however, is often understood and described relative to the human visual system, where most individuals discuss it in terms of its apparent color and brightness.

This description of light is serviceable for purposes of general communication, but is less useful as a descriptor in photobiology. There are two broad categories of light measurement techniques: radiometric and photometric (19). Radiometry is based exclusively on the physical properties of light, such as its energy and wavelength, whereas photometry is based on the selective responsiveness of the human visual system (19,20). Radiometric quantification of stimuli is particularly important in photobiological research. A radiometer measures the radiant power of a light source over a defined range of wavelengths, while a photometer measures the luminous flux falling on a surface that stimulates the human eye during the daytime. A photometer is simply a radiometer that has filters added to the detector which “shape” the detector sensitivity to resemble the luminance (brightness) response of the human visual response of the “standard observer” as determined by the CIE (20). Thus, photometry is a special branch of radiometry. Although the photometric system provides a serviceable nomenclature and measurement technique for describing the light stimuli used in this manuscript, it does not imply that the investigators accept that lux measurements are specifically relevant to circadian and neuroendocrine responses in rats. To the contrary, the photoreceptive physiology in rodents for circadian and neuroendocrine regulation has not been identified (21). It is very unlikely that the visual sensitivity of the human eye will be equivalent to the sensitivity of the input physiology of the rodent circadian system. To compare our results relative to national standards for specifying light in animal housing facilities (7), we have framed the studies reported here in terms of photometry. We have also reported our light stimuli in radiometric terms ( $\mu\text{W}/\text{cm}^2$ ) for greater utility to the research community. The

optimal measurement for circadian and neuroendocrine regulation in animals will only be resolved when the photobiological physiology which mediates these effects of light is clarified.

Light suppression of pineal gland melatonin was used as a bioassay in this study because it is generally accepted as one of the most sensitive measures of the effects of light on the circadian system of rodents and other species (22). The exposure of the mammalian retina to light during the night induces neuronal signals in the retinohypothalamic pathway, which in turn elevates glucose metabolism in the suprachiasmatic nuclei of the hypothalamus (23). Signals from the suprachiasmatic nuclei are transmitted to the pineal gland which leads to rapid suppression of pineal enzymes and subsequent lowering of pineal gland melatonin (24). The suprachiasmatic nuclei have been known to be the endogenous pacemakers for circadian rhythms in mammals, including the rhythmic pineal gland synthesis and secretion of melatonin (25). This response of the retinal-hypothalamic-pineal axis to light is dependent on the intensity (26) and wavelength (27) of the light. Therefore, the ability of each light source to suppress pineal gland melatonin at each of the light intensities used provided a meaningful dose-response comparison between LED light and CWF light. The data from these experiments indicate that there is no significant difference in melatonin suppression between rats exposed to CWF light and rats exposed to LED light at illuminances ranging from 100 lux to 0.1 lux. The significant suppression at the lowest illuminance suggests that even lower intensities of CWF and LED light might suppress pineal gland melatonin.



The sensitive nature of the retinal-hypothalamic-pineal axis in the white laboratory rat was initially demonstrated by Minneman and colleagues (28). The ability of LED light to suppress pineal gland melatonin concentrations significantly at low illuminances, comparable to that of the CWF light, suggests that it can be equally effective in regulating other aspects of circadian system physiology. Further tests on other circadian parameters are required to confirm this.

Light exposure can cause retinal damage and irreversible loss of photoreceptor cells, causing partial or total blindness (2,3,4,29,30). It was deemed especially important to determine whether the LED arrays caused phototoxicity because of the narrow wavelength band in LEDs as light damage is produced more readily by some wavelengths of visible light (2). To determine whether the use of LED lighting in animal habitats would have any deleterious effect on the retina, we compared effects of LED animal habitat lighting and CWF animal habitat lighting on retinal function using ERG assessment. The ERG is the evoked mass response of the retina to a flash of light (31). The amplitudes of the individual components of the composite ERG waveform can be related to specific cellular layers of the neural retina and the retinal epithelium (31). The a-wave is the first part of the ERG and is the component produced by the photoreceptor cells (31). The b-wave, which is the most salient fast potential of the ERG of the intact retina, is a result of changes in extracellular potassium ion concentrations in Muller cells as a result of on-bipolar excitation (31). Loss of retinal function would be indicated by the reduction in amplitude of the ERG a- and b-waves, showing a reduced sensitivity to

light or functional loss. In addition to loss of visual function, retinal phototoxicity may also affect the hypothalamic-pineal axis due to the transmission of signals from the eye to the central pacemaker of physiological and behavioral rhythms. The results of the ERG assessment show that LED does not reduce photoreceptor sensitivity nor disrupt retinal function (Figures 4 to 7).

Morphological assessment was done following the ERG functional assessment in order to determine the presence of any photoreceptor cell damage or death. Past findings have shown photoreceptor degeneration to occur in albino rats exposed to 60 lux light using a 12:12 LD cycle (32). The histological data obtained in this study support the conclusion that chronic 100-lux LED lighting does not cause retinal damage (Table 1).

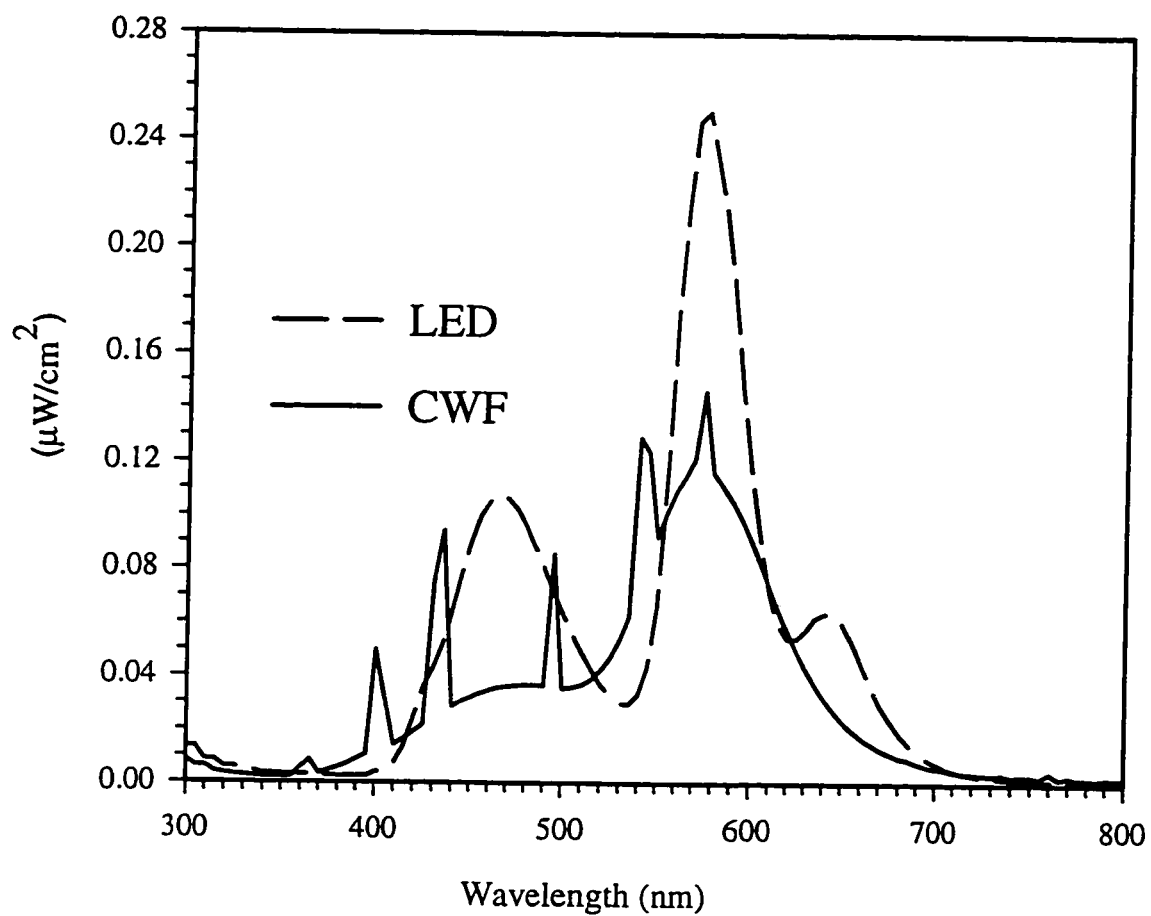
These data indicate that LED light may be a suitable alternative to CWF light for animal habitat lighting. LED light appears to support normal circadian physiology and caused no morphological destruction in the retina. Replacing incandescent or fluorescent light sources with LEDs carries a number of advantages including longer operating life, less mass and volume, less heat production, less power consumption and higher efficiency (candela/watt of power)(8). In addition to the mechanical and economical advantages, LEDs can be used to produce more precisely timed and spectrally controlled photic stimuli.

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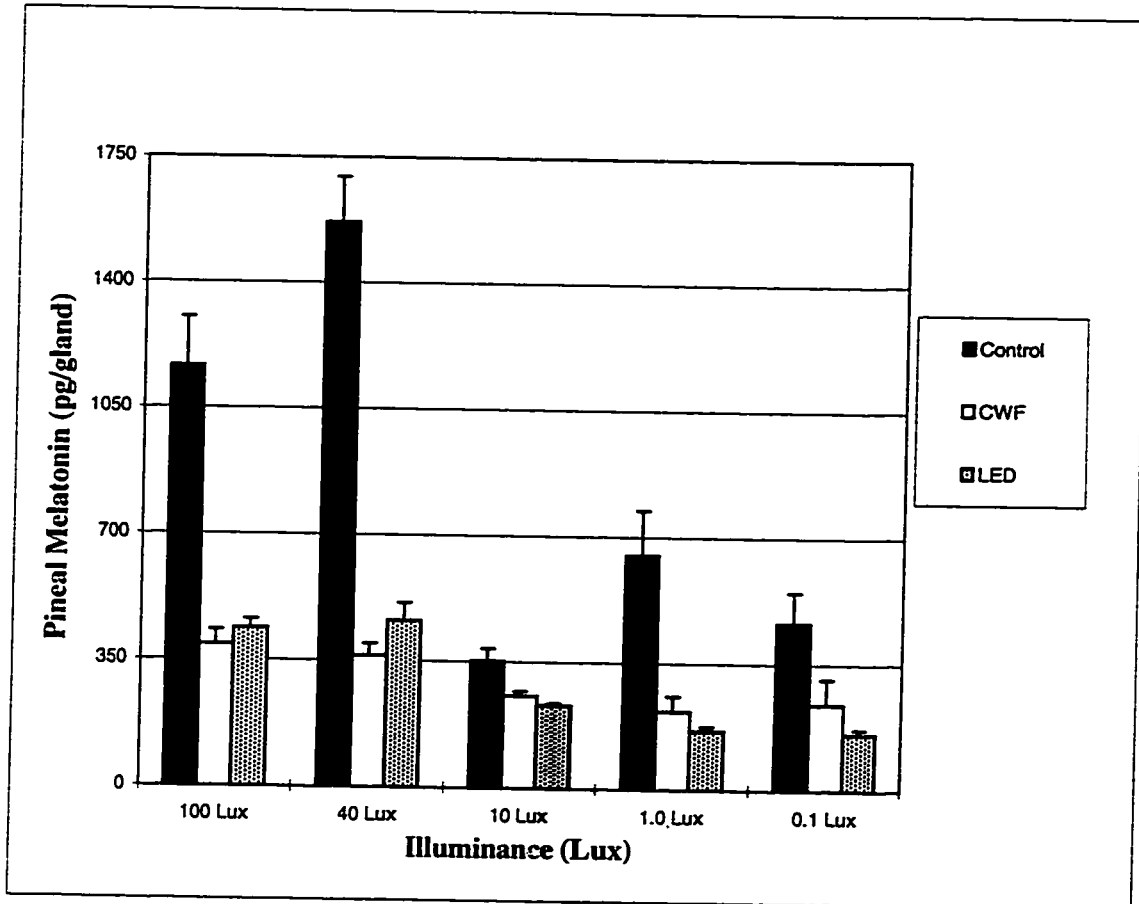
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**Figure 1.** Spectral power distribution curves of LED and CWF light sources used in this study. Energy distribution for LED array is as follows: blue (35.8%), green/yellow (47.2%) and red (17%). Energy distribution for CWF light source is as follows blue (34.6%), green/yellow (48.6%) and red (16.8%). Bandwidths with corresponding wavelength ranges and photon energies are as follows: Blue (460-490nm/2.53-2.70eV), Green (490-575nm/2.16-2.53eV), Yellow (575-585nm/2.12-2.16eV) and Red (760-610nm/1.63-2.03eV).



**Figure 2.** Pineal melatonin suppression in control (dark adapted), LED-exposed and CWF-exposed rats at 5 different light illuminances. Values are mean  $\pm$  SEM, n=8 per group. Melatonin was significantly reduced by light exposure but there were no differences in melatonin between CWF and LED animals. Abbreviations: Light Emitting Diode (LED), Cool White Fluorescent (CWF).

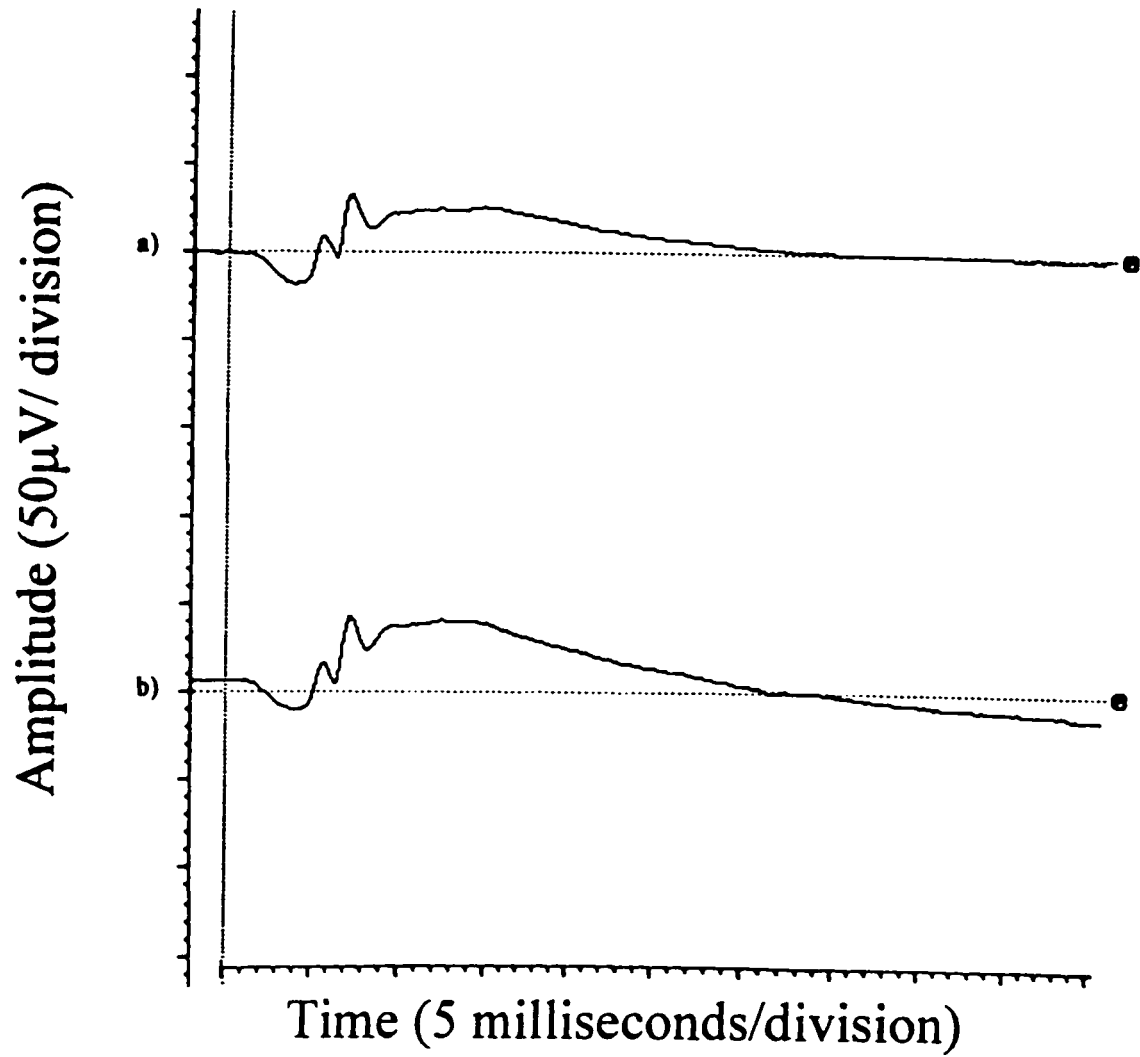
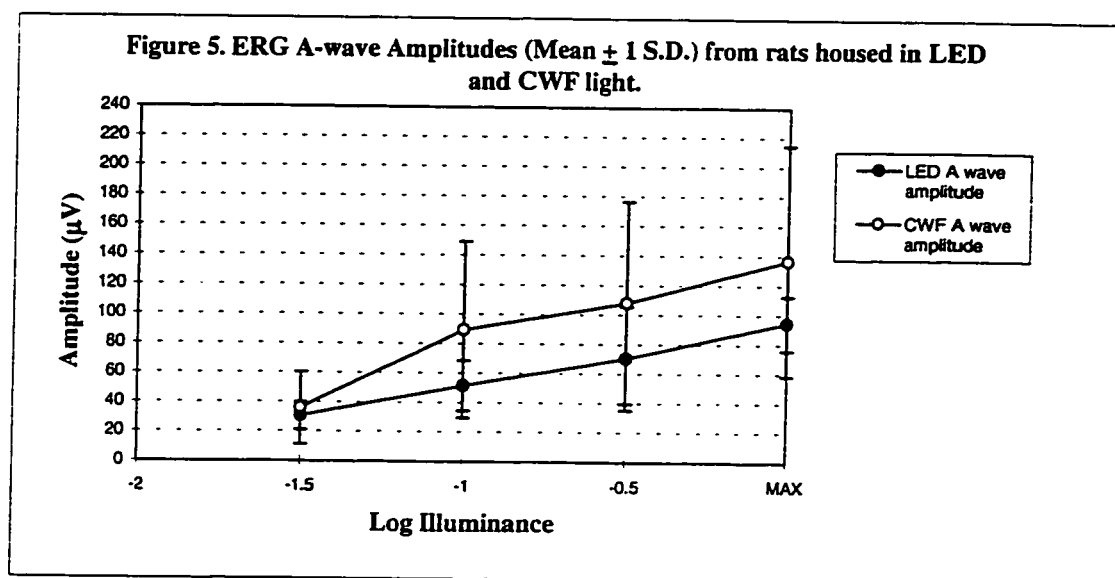
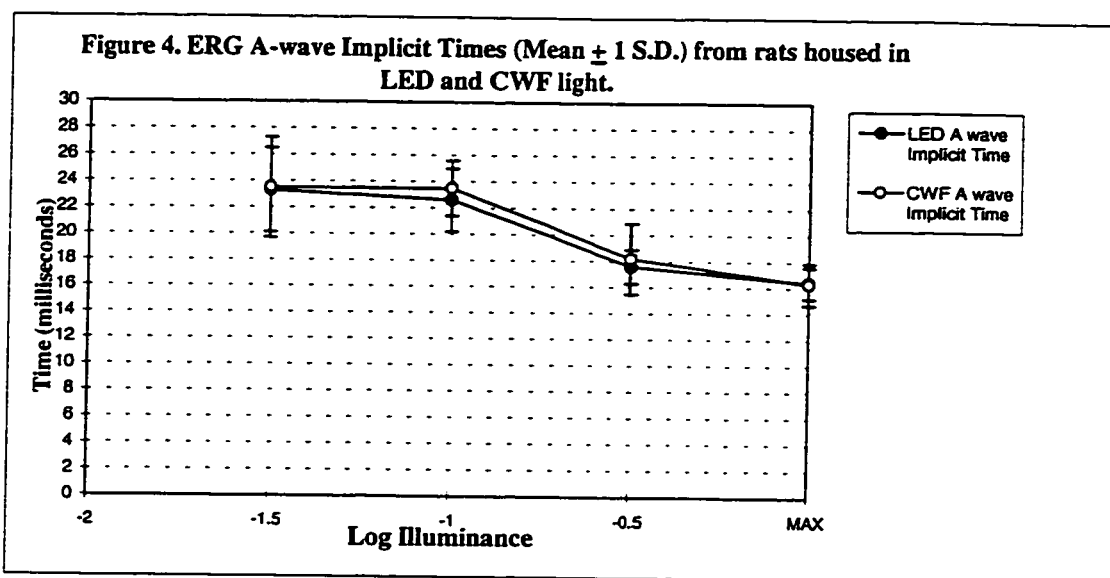
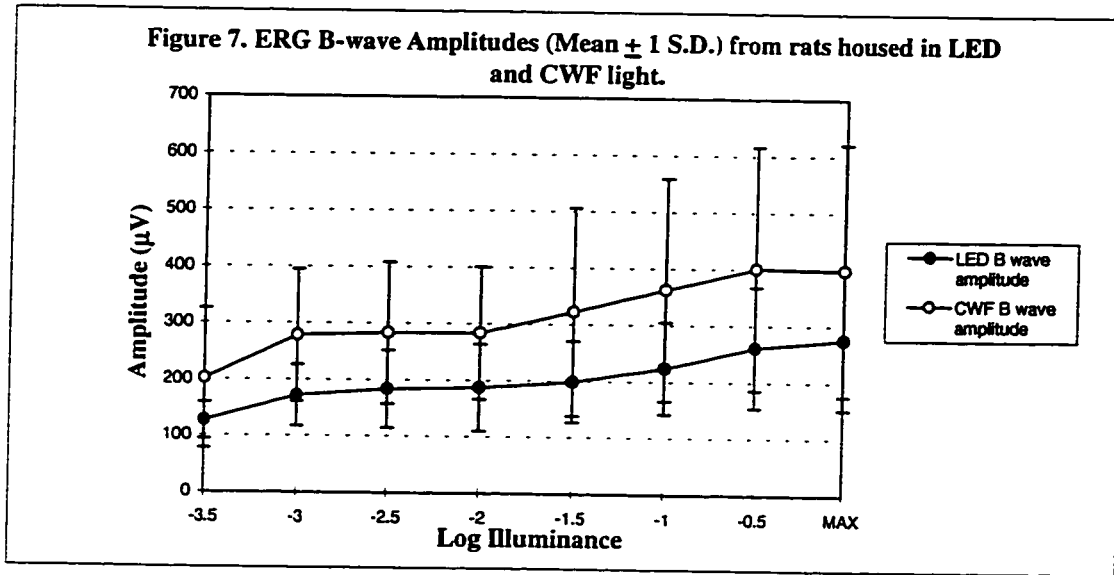
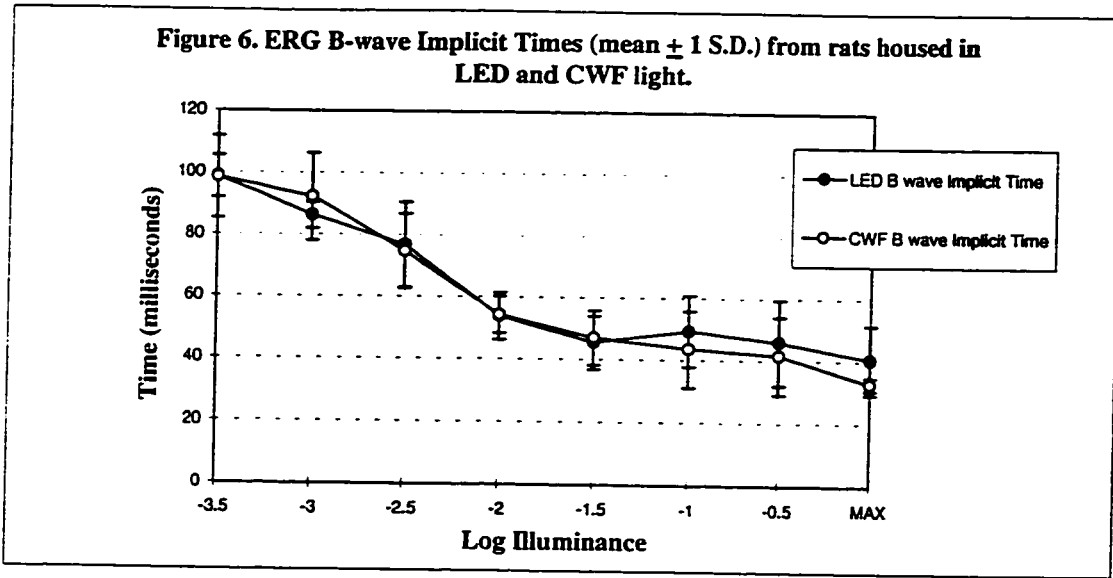


Figure 3. ERG waveforms evoked (obtained with a monochromatic blue stimulus) from a rat housed in CWF light (a) and a rat housed in LED light (b).



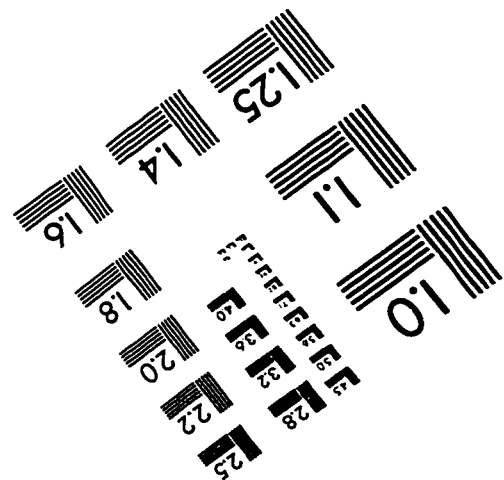
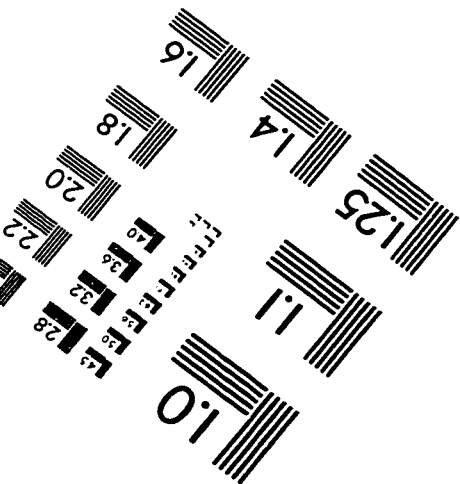
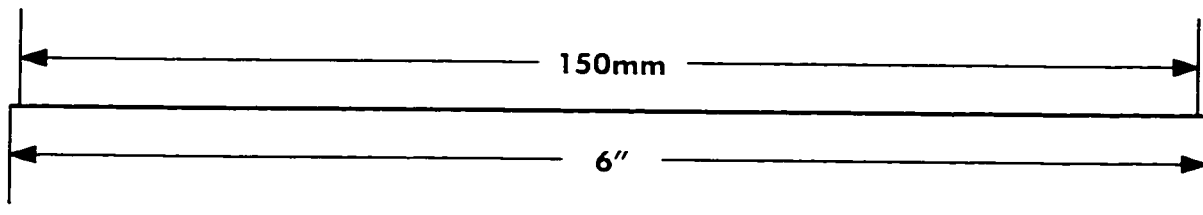
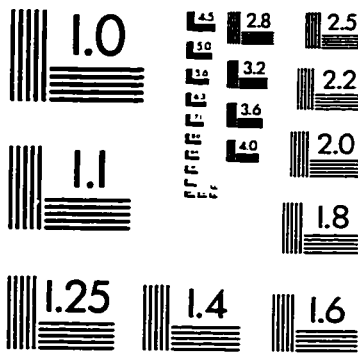
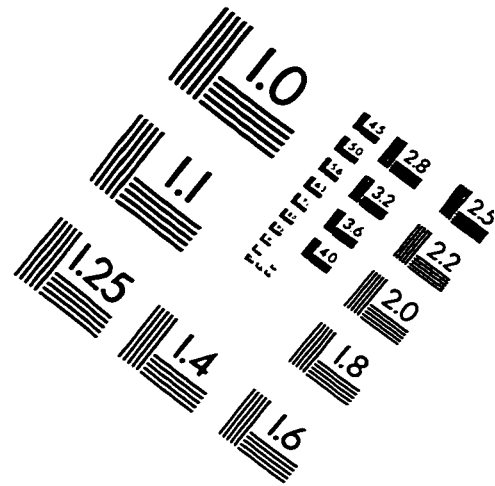
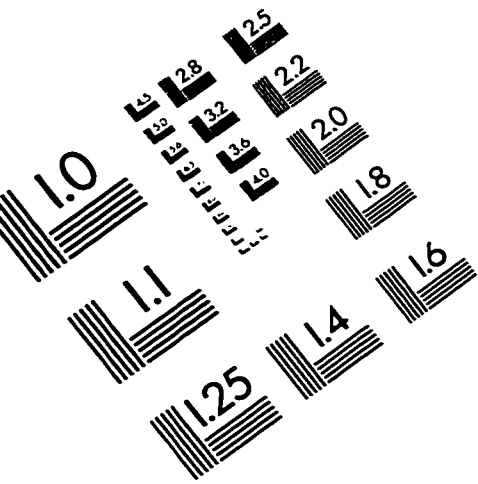




**Table 1. Summary of retinal histology (mean  $\pm$  1 S.D.) performed on LED-housed rats (n=7), CWF-housed rats (n=7), and Control rats (n=3). ANOVA performed on data with p values shown.**

	<b>Control</b>	<b>CWF</b>	<b>LED</b>	<b>p value</b>
Rod Outer Segment Length ( $\mu\text{m}$ )	24.38 $\pm$ 4.51	24.20 $\pm$ 6.35	22.14 $\pm$ 3.20	0.69
# Rod Nuclei, outer nuclear layer	9.67 $\pm$ 0.80	9.32 $\pm$ 0.97	9.00 $\pm$ 0.88	0.56
Retinal Thickness ( $\mu\text{m}$ )	188.33 $\pm$ 27.88	210.8 $\pm$ 42.48	186.07 $\pm$ 32.10	0.43

# IMAGE EVALUATION TEST TARGET (QA-3)



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